CLAIMS

What is claimed is:

1. A method for identifying a cell-specific cis-regulatory element, comprising the steps of:
    a) providing a first host cell population with a first set of reporter constructs comprising a first library of nucleic acid fragments, each operatively linked to a reporter gene;
    b) selecting from said first host cell population a first cell subpopulation having a first level of reporter activity;
    c) providing a second host cell population with a reporter construct comprising a first sub-library of nucleic acid fragments recovered from said first subpopulation;
    d) counterselecting from said second host cell population a second subpopulation having a second level of reporter activity; and
    e) recovering from said second subpopulation a second sublibrary of nucleic acid fragments;

wherein said second sublibrary comprises at least one cell-specific cis-regulatory element.

2. The method of claim 1, wherein said cell specific element is cell-type specific.

3. The method of claim 1, wherein said first host cell population and said second host cell population are developmentally-related cell types.

4. The method of claim 3, wherein said developmentally related cell types are selected from the group consisting of breast cancer cells vs. normal mammary epithelial cells, lung cancer cells vs. normal lung epithelial cells, colon cancer cells vs. normal colon epithelial cells, ovarian cancer cells vs. normal breast cells, melanoma cells vs. normal melanocytes, leukemia cells vs. normal leukocytes, prostate cancer cells vs. vs. normal prostate cells, an metastatic vs. non-metastatic cells.

5. The method of claim 1, wherein said cell-specific element is cell-state specific.

6. The method of claim 1, wherein one of said first host cell population and said second host cell population is a growth-arrested population, and the corresponding other population is non growth-arrested.

7. The method of claim 1, wherein one of said first host cell population and said second host cell population is responsive to a preselected agent, and the corresponding other population is non-responsive to said pre-selected agent.

8. The method of claim 7, wherein said preselected agent is selected from the group consisting of retinoic acid, estrogen, insulin, progesterone, growth factors, cytokines and nutrients.

9. The method of claim 1, wherein said cis-regulatory element is active in mammalian cells.

10. The method of claim 9, wherein said first and said second host cell populations are mammalian cell populations.

11. The method of claim 10, wherein at least one of said mammalian cell populations is a cancer cell population.

12. The method of claim 11, wherein said cancer cells are selected from the group of melanoma, breast cancer, colon cancer, ovarian cancer, leukemia and prostate cancer.]

13. The method of claim 1, wherein said first and second host cell populations are plant cell populations.

14. The method of claim 1, wherein said first and second host cell populations are microbial cell populations.

15. The method of claim 1, wherein said selection and counterselection steps comprise FACS analysis.

16. The method of claim 1, wherein said reporter construct is a fluorescent reporter construct.

17. The method of claim 16, wherein said fluorescent reporter construct is GFP.

18. The method of claim 1, wherein said first library of nucleic acid fragments is genomic DNA.

19. The method of claim 1, wherein said first library of nucleic acid fragments are nucleic acids synthesized in vitro.

20. A method for identifying cell-type specific cis regulatory elements, comprising the steps of:

(a) generating a library of nucleic acid fragments in an expression vector comprising a sequence encoding a reporter molecule;

(b) introducing the library into a plurality of first host cells;

(c) selecting from the plurality of first host cells one or more library-c containing first host cells having predetermined level of reporter gene e expression;

(d) recovering from the selected library-containing first host cells a sub-library of nucleic acid fragments;

(e) introducing the sub-library into a plurality of second host cells;

(f) selecting from the plurality of second host cells one or more sub-library-containing second host cells having a second predetermined level of r reporter gene expression; and

(g) recovering the sub-library fragments from the selected second host cells.

21. The method of claim 20, further comprising reintroducing the sub-library fragments recovered in step (g) into the plurality of first host cells, and repeating steps (c) through (g).

22. The method of claim 20, further comprising reintroducing the sub-library fragments recovered in step (d) into the plurality of first host cells, and repeating step (c).

23. The method of claim 20, further comprising reintroducing the sub-library fragments recovered in step (g) into the plurality of second host cells, and repeating step (f).

24. The method of claim 20, wherein the steps of selecting comprise the use of a fluorescence activated cell sorter.

25. The method of claim 21, wherein the recovered sub-library fragments are manipulated in vitro prior to the reintroducing step.

26. A method for characterizing one or more protein factors that bind to an identified cell-type specific cis regulatory element, comprising the steps of:

(a) preparing an extract containing the factors;

(b) incubating the extract with the identified cell-type specific cis regulatory element under conditions in which the factors specifically bind to the cis regulatory element; and

(c) substantially purifying the specifically bound factors.

27. A method for identifying a novel host cell sequence variant, comprising the steps of:

(a) stably propagating a cell-type specific cis sequence operatively linked to a reporter in a population of host cells;

(b) selecting a sub-set of host cells in which the reporter expression level differs from the average reporter expression level in the host cell population; and

(c) isolating individual host cells from the selected sub-set.

28. The method of claim 27, further comprising the steps of:

(d) expanding a new population of host cells from the individual host cells isolated from the selected sub-set;

(e) selecting a second sub-set of host cells in which the reporter expression level differs from the average reporter expression level in the new population of host cells; and

(f) isolating individual host cells from the selected second sub-set.

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